REMARKS/ARGUMENTS

After entry of this amendment Claims 67, 69-73 and 75-87 will be pending and under examination. Cancelation or amendment of claims is without disclaimer or prejudice to future prosecution.

Claim Amendments

Claim 67 is amended to more clearly describe the invention. For the convenience of the Office, the claim as amended is shown below.

> 67. A method of obtaining nucleic acid from a sample of heterogeneous biological elements, the method comprising:
>
> (a) providing a sample comprising heterogeneous biological elements, wherein said elements are cells and/or virions;
>
> (b) physically isolating an individual biological element or a subset of the biological elements of the sample within a microfluidic flow channel wherein the isolation step comprises allowing the individual biological element or the subset of the biological elements to flow past a first valve of the microfluidic device, creating a segment within the flow channel by actuating the first valve and a second valve of the microfluidic device, and preventing the individual biological element or the subset of the biological elements from exiting the segment past the first valve or the second valve;
>
> (c) lysing the individual biological element or the subset of biological elements thereby releasing nucleic acids from the biological element(s); and
>
> (d) amplifying at least a portion of the nucleic acid,
>
> wherein steps b, c, and d are performed in a microfluidic device.

In (a) it is specified that the biological elements are cells and/or virions. The cells may be prokaryotic or eukaryotic. In (e) the extraneous term "purified" is removed as lacking antecedent basis. As known in the amplification art, and noted in the specification at, e.g., paragraphs [0472] and [0473], amplification does not necessarily require nucleic acid purification. (Paragraph numbers refer to US 20050053952.)

Claims 70 and 73 are similarly amended. Claims 69 and 72 are amended to increase clarity.

Rejections Under 35 U.S.C. §103

Claims 67-69, and 76-79 were rejected under 35 U.S.C. 103(a) as allegedly obvious in view of US Pat. No. 6,007,690 ["Nelson"] and US Pat. No. 6,408,878 ["Unger"]. Applicants respectfully traverse this rejection.

Nelson described transporting a sample, such as a cell lysate or cell population, through an enrichment channel with a solid phase extraction (SPE) chamber to isolate a target analyte or subpopulation of cells. Cells or analytes are selected based on binding of the target analyte or cell to an enrichment medium (*See* Nelson, Col. 5 and 6). In one example, Nelson described selection of a subset of cells from a heterogeneous mixture of cells. *See* Nelson Example 4, relied on by the Office.

The Office acknowledges that Nelson did not describe a method of isolating a subpopulation of cells from a heterogeneous population, followed by lysis of the isolated cells to release nucleic acids, followed by amplification of released nucleic acids. However, the Office asserts these steps are implicit because Nelson states that RT-PCR may be carried out using the isolated cells, and this would necessarily require the disruption of cells.

The Office also acknowledges Nelson did not describe allowing elements to flow past a first valve and actuating the first valve and a second valve thereby preventing the biological elements from exiting that segment of the flow channel. However, the Office asserts that Unger et al. remedies this deficiency.

Applicants respectfully disagree.

The processes described in Nelson are based on conventional purification methods. A target analyte, or target cell identified by a characteristic marker, is absorbed to or immobilized on an insoluble matrix via a selective "binding member" (e.g., anti-ligand) that binds the analyte or cell marker, while unbound (i.e., unselected) molecules or cells are washed away.[1] In Nelson, isolation of cells is based on selectable properties of the target cells, such as the presence of a cell surface marker. The Office characterizes this as "separation and selective retaining of target analytes" (Office Action at page 3, lines 4-5).

In contrast, in the present invention, cells are not selected based on binding of an anti-ligand or affinity to a matrix, or any method described or suggested by the Nelson reference. Rather, in the present invention one or more cell and/or virions is isolated by partition in a microfluidic channel. While Nelson described isolation or analytes or cells according to a shared property, the biological elements isolated according to the present invention may include combinations of cells and/or virions that do not share a particular characteristic ligand recognized by a binding agent. Conversely, cells or virions that do share a particular characteristic ligand may be (and typically are) isolated in separate, fluidically distinct, channel segments. The process, goal and effect of the current method is unlike the conventional separation methods described in Nelson.

The Office recognizes that Nelson and the present invention differ, but argues that Unger provided motivation to modify Nelson. Specifically, the Office characterizes the methods of Nelson as "sample retention" and asserts that Unger suggests the use of "valves and pumps" in "sample retention and control." In support of this, the Office quotes at length from a description in Unger of the use of valves and pumps as substitutes for electro-osmotic flow systems.[2] The position of the Office is flawed in at least two basic respects. First, the Office incorrectly equates "sample retention" (as described in Nelson) with "flow systems" (as described in Unger). If an analogy is to be made, it is between the "electrophoretic flowpath" described in Nelson. (*See* Col. 4, lines 1-17) and the valve and pump based "flow systems" in Unger. Contrary to the assertion of the Office Action, the Unger reference would not have suggested substituting valves and pumps for Nelson's enrichment medium.

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[1] E.g., absorption of nucleic acids to beads, hybridization of nucleic acids to immobilized complementary polynucleotides, electromagnetic isolation of cells using Dynabeads "capable of binding a specific target" and the like. See, for example, Col. 23, line 55 to Col. 24, line 28. Other binding media, for example metal chelating agents, and the like may be used in the Nelson method, although such alternative methods do not seem suitable for cell isolation. See Col. 5-6.

[2] See Office Action, pages 4-5.

Second, the Office incorrectly equates affinity-based selection of cells or molecules in a solution (Nelson), with physical segregation or partition of the solution itself, as in the present invention. These elements simply cannot be substituted for each other. Indeed, not only are they different processes, they lead to different results: The current invention may result may result in isolation of combinations of biological elements that do not share a particular property and conversely may result in separation of identical biological elements away from each other. Moreover, the current invention allows isolation of an individual biological entity, not possible in an affinity binding system.

Thus, Nelson describes selection based on a property of the target cells or analytes. The present invention does not use such selection. Nothing in the Unger reference would have provided one of skill in the art with a motivation to modify Nelson to eliminate the selection step characterized by Nelson as "critical", and substitute a wholly different process that produces a qualitatively and quantitatively different result. Indeed, the substitution suggested by the Office would render the Nelson method nonfunctional. See Nelson Col. 4, lines 43-53.

Burlage (US 2003/0099928) is cited by the Office along with Nelson and Unger as allegedly making Claims 70-73, 75, and 80-87 obvious. The Office acknowledges that Nelson and Unger do not suggest a method of characterizing phylogenetic, gene, and functional diversity exhibited by a specific environment. Burlage describes isolating microorganisms by (1) labeling fixed cells based on hybridization of a fluorescently labeled polynucleotide probe and (2) separation of fixed cells using fluorescence-activated cell sorting.

The Office proposes it would have been *prima facie* obvious to combine the teachings of Nelson et al. and Burlage because of the advantages of combining assays on a single microfluidic device (Office Action, page 7). Assuming, *arguendo*, this is correct, Applicants respectfully submit this begs the question of why one would have been motivated to combine Burlage's FACS-based separation and Nelson's affinity-based separation. In fact, the methods cannot be combined: Burlage requires internalization of the labeled probe, while Nelson provides no mechanism for selecting cells based on an internalized probe.

Moreover, Burlage clearly does not remedy the deficiencies of Nelson and Unger as discussed above.

Finally, Applicants respectfully request clarification of the rejection as it applies to Claims 76, 79, 80, 84 and 87, should any rejection be maintained. Applicants appreciate the comments of the Office at page 10, second full paragraph. However, Claims 76, 80 and 84 are directed to introducing a lysing chemical through *a cross-flow injector*. This has not been addressed by the Office and is not suggested in the cited references. With regard to Claim 87, the Office states Unger discloses "lysis in a rotary mixer." The Office does not state where such a disclosure is found in the reference and Applicants are not able to find any reference to "lysis" or "rotary" in the reference.[3]

Interview

To expedite prosecution, Applicants respectfully request an interview with the Examiner at a mutually convenient time. Applicants representative will contact the Examiner to arrange such an interview.

CONCLUSION

In view of the foregoing, Applicants believe all claims now pending in this Application are in condition for allowance and an action to that end is respectfully requested.

If the Examiner believes a telephone conference would expedite prosecution of this application, please telephone the undersigned at 650-462-5330.

Respectfully submitted,

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[3] Claim 79 also recites a rotary mixer.